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Short Communication

New Results on the Chemistry of Metal-Tetrahydro-Pterin Complexes

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Introduction

The synthesis of trichloro(6β-quinonoid-6,7-dihydro(8H)-L-biopterin)oxomolybdenum(IV) **1**, the first molybdenum complex of a quinonoid dihydropterin, has enhanced the interest in metallo-hydrogenated pterin-complexes. These may act as coenzymes of oxidoreductases in numerous biological reactions (2, 3).

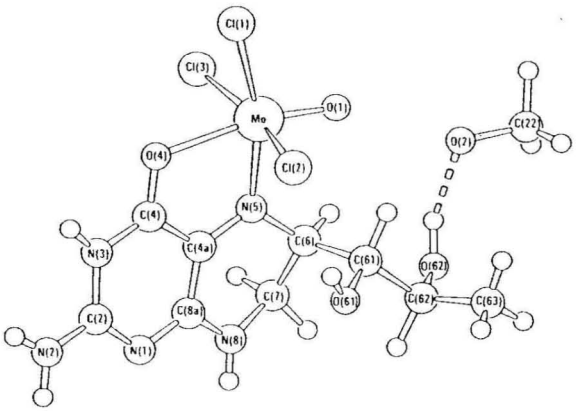

Figure 1. Crystal structure of **1**.

It is difficult to obtain product **1** in high yields, because 6β-5,6,7,8-tetrahydro-L-biopterin (BH_4) is expensive; therefore we tried to obtain an analogous product with a pterin, that is easier to synthesize in large quantities. We chose tetrahydropterin as a starting material, which is not chiral and can be obtained very pure.

Part 93 (1)

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Tetrahydropterin **3a** reacts with $Mo^{VI}O_2Cl_2$ in the same fashion as BH_4 **3b** to give trichloro(quinonoid-6,7-dihydro(8H)pterin)oxomolybdenum(IV) **2**, which we were able to crystallize. The crystal structure of **2** is very similar to that of **1** in respect to bond

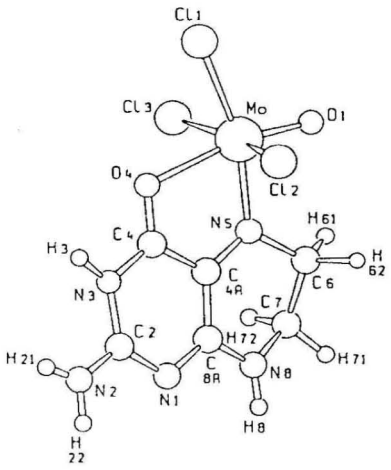
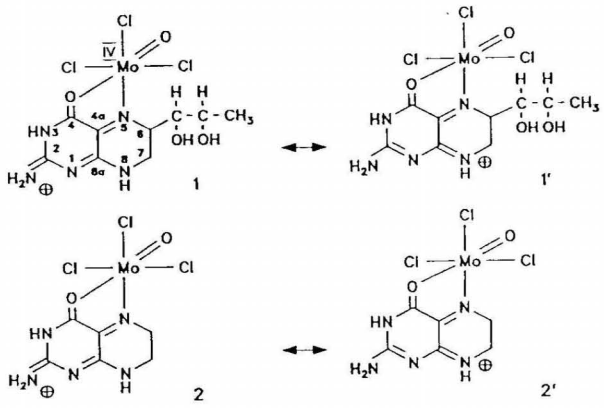


Figure 2. Crystal structure of **2**.

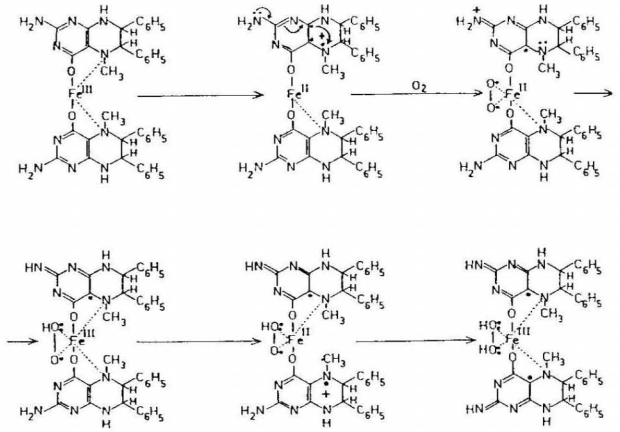
length, angles, and conformation (Figures 1 and 2).

The only difference in the crystal structures is the presence of a solvent molecule (methanol) in **1** which is absent in the crystal of **2**.

Analysis of bond lengths and angles in both crystal structures allow us to assume that we obtained quinonoid dihydropterins coordinating a molybdenum(IV) atom with the atoms O(4) and N(5). The quinonoid dihydropterins are monoprotonated at N (2′) and possess a N(2′)-N(8) mesomeric system (**1**↔**1′** and **2**↔**2′**) (Scheme 1).



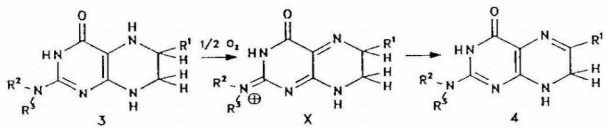
Scheme 1.



Scheme 2. (4)

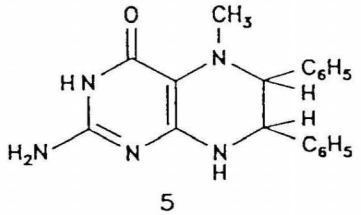
In addition one can note that a C(6)-sidechain is not necessary for the formation of a complex.

These properties allow one to use tetrahydropterin **3a** or some of its derivatives as good model compounds for the study of metal-hydropterin coenzymes of oxidoreductases like phenylalanine hydroxylase. Furthermore one should be able to study the metal-N(5)-methyl-tetrahydropterin coenzymes like homocysteine-methionine methyltransferease.



	R^1	R^2	R^3
a:	H	H	H
b:	H H C - C - CH_3 OH OH	H	H
c:	H	CH_3	CH_3

More than 20 years ago, we were interested in a N(5)-methyl-6,7-diphenyl-tetrahydropterin-iron(II) or-iron(III) complex **5**-iron complex). At that time it was easy to obtain **5** and we observed the formation of a coloured compound when mixing together $FeCl_3$ and **5** (4).



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In the same paper (4) we published a theoretical concept for the formation of a quinonoid-dihydropterin-iron complex, in which we summarized the results of our research on the oxidation of tetrahydropterins with O_2 (Scheme 2):

1. Necessity of Fe(II) or Fe(III) together with ethylenediaminetetraacetic acid (EDTA) as ligand for the activation of O_2 by tetrahydropterins (5).

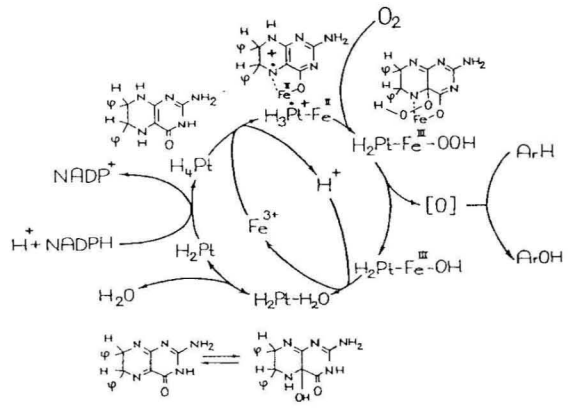
2. Demonstration that the oxidation of a tetrahydropterin **3** to the 7,8-dihydropterin **4** does not occur in one step. Using special conditions we could show, that an intermediate product **X** is formed. **X** is only stable if N(2′) is disubstituted as it is the case with N(2′),N(2′)-dimethyl-tetrahydropterin **3c**. In analogy to *p*-dimethylamino-phenylene-diamine and its *p*-quinonoid structure, we proposed **Xc** to be a relatively stable quinonoid cation (6).

Shortly before, Kaufman showed that during the enzymatic hydroxylation of phenylalanine to tyrosine BH_4 **3b**, which acts as coenzyme of the hydroxylase, is not directly oxidized to 7,8-dihydro-L-biopterin **4b**. He proposed the intermediate **Y**. During the discussion of this paper at the 3rd Symposium on pterin chemistry in Stuttgart, Hemmerich suggested that **Y** could be a quinonoid-dihydropterin **Xb**; kaufmann agreed to this suggestion (7).

3. Presentation of a theory implying the formation of radical cations during the oxidation of tetrahydropterins (8).

4. We proposed that in the presence of Fe(II) or Fe(III) and EDTA, during the oxidation of **5** to a dihydropterin derivative, an addition of one oxygen atom to the position 4a in **5** takes place (9).

5. Finally we obtained experimental proof for the formation of quinonoid trihydropterin radical cations during the oxidation of **5** with H_2O_2 (10). In the same paper we published a possible mechanism of the enzymatic hydroxylation of phenylalanine to

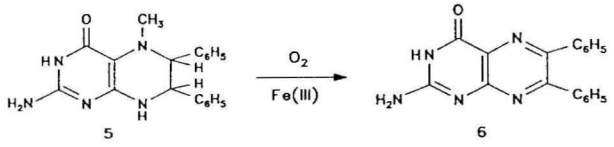


Scheme 3. (10)

tyrosine in the case of a mixed oxidation (Scheme 3).

Two years later at the 4th Symposium of Chemistry and Biology of Pteridines we extended this mechanism to the situation of a non-mixed oxidation (11).

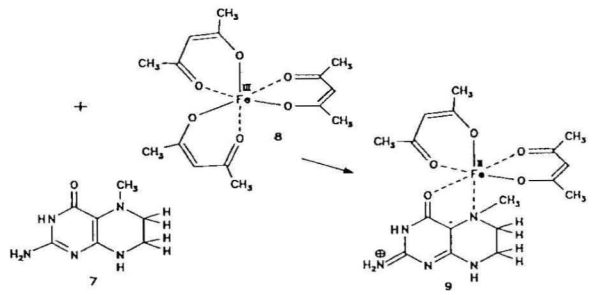
In all experiments using **5** we observed a loss of the N(5)-methyl group during the oxidation. Only 6,7-diphenyl-pterin **6** was isolated as the final product (12).



Since it is reasonable to replace BH_4 by tetrahydropterin in the formation of metal complexes with molybdenum, we started to repeat the experiments mentioned above with N(5)-methyl-tetrahdropterin **7** and a Fe(III) complex of the composition $Fe(acac)_3$ **8** (acac=acetylacetonate). Without H_2O_2 as an oxidant, we hoped to find an experimental confirmation for the existence of the radical cations, which we proposed 25 years ago.

N(5)-methyl-tetrahydropterin **7** can be obtained as a pure and very stable dihydrochloride in a simple fashion by a small modification of Matsuura's method (13). The important step was to find a way to detect the complex formed between **7** and **8**.

An electrospray-ionisation-mass spectrum (ESI-MS) in the positive ion mode was run of compound **7** ·2 HCl in a 10^{-3} M solution at pH 2-3 in methanol or a mixture of acetonitrile and water. Both show the $[M+G]^+$ signal at m/z 182 of the protonated starting material **7** as well as other protonated molecule clusters of **7**. The formation of clusters containing up to seven molecules of **7** are most likely to be due to the high concentration of the solution. This is in accordance with analogous experiments with arginine (14).



The ESI-MS of a 1:1 molar solution of **7** and **8** show protonated clusters of **7** and **8** and a new signal at m/z 435, which corresponds most likely to complex **9**. Arguing from analogy with complexes **1** and **1'** one could propose complex **9** to be a radical cation.

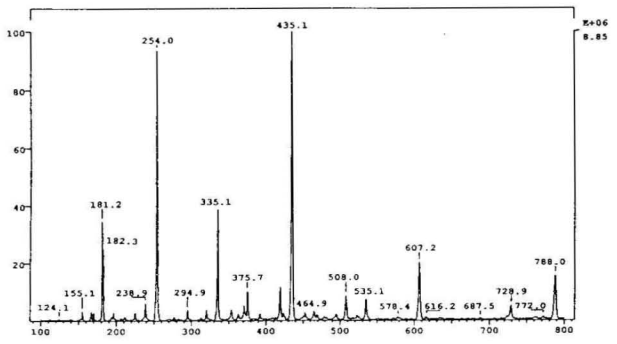
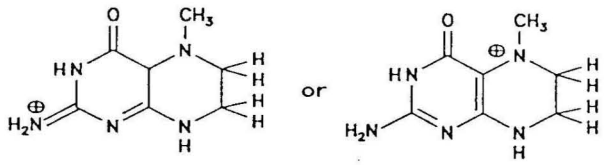


Figure 3. Electrospray-ionisation-mass spectrum of **7** and **8**, in a 1:1 molar ratio in acetonitrile/water, pH 2-3. The signal at m/z 435 corresponds to the proposed radical cation complex **9**.

So far the best conditions for the formation of the metal complex **9** with a molecular weight of 435 are strict exclusion of oxygen and a 1:1 molar ratio of **7** and **8** in acetonitrile with the smallest amount of water necessary to keep **7** in solution (Figure 3).

Figure 4. ESR spectrum 1:1 molar solution of **7** and **8** after 24 hours under argon, acetonitrile/water, pH 2-3.



Cationic trihydropterin radical **10**

In the absence of oxygen compound **9** is stable for more than 24 hours. Even after this time the solution shows the ESR signal of a stable radical. The spectrum corresponds to known ESR signal of a cationic trihydropterin radical **10** formed by H_2O_2 oxidation of tetrahydropterin (G value=2.003, splitting ca. 10 G) (10, 15) (Figure 4).

A full paper will be published in *Helvetica Chimica Acta*.

Acknowledgement

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